METHOD DEVELOPMENT FOR GREEN COFFEE ANALYSIS AND ITS POSSIBLE APPLICATION FOR GROUP DISCRIMINATION AND CORRELATION OF GREEN COFFEE CHEMISTRY WITH CUPPING QUALITY

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1
INTRODUCTION

Coffee has been studied in Hawai'i for over 70 years. The type of research varied depending on the needs at that time. In the 1930's and 1940's, researchers primarily focused on pests, nutrition and pruning. An abundant amount of research occurred in the 1950's and 1960's. In general, the work related to pruning, fertilization, mechanical harvesting and processing. By the end of the 1960's through the early 1980's, very little coffee research transpired, primarily as the coffee industry was in decline.

Up to this point, coffee research dealt with crop production and processing. In the mid-1980's, a new aspect of coffee was explored- quality. A statewide trial was started to evaluate locations and genotypes and their effects on coffee quality (Farmer's Bookshelf, 2002). Various aspects of coffee science were studied in the 1990's. The major topics included the greenscale-ant complex, a nematode pest in Kona, pesticide registration, flowering control, water relations, mechanized pruning, genetic engineering and, again, quality. A breeding program aimed at producing a unique, high quality coffee variety for Hawai'i was started (Nagai et al., 2001). This thesis aims to further explore coffee quality.

Coffee is an important crop worldwide; it is produced in over 50 countries. In recent years, coffee production has increased at a faster rate than coffee consumption. As a result, world coffee prices have plummeted.

An effective method of maintaining or establishing high prices in a free market is to distinguish a particular coffee so that consumers become willing to spend more money for that coffee. Name recognition is effective for maintaining high prices as evidenced

by Hawai'i's Kona Coffee and Jamaica's Blue Mountain Coffee. Both of these command a price that is at least double that of the world's other specialty coffee.

Name recognition alone will not sustain world coffee prices. A more effective method is to produce high quality coffee and have the ability to prevent fraudulent behavior by individuals making inaccurate claims about certain coffees. Accomplishing this goal necessitates that farmers produce high quality coffee and that an objective means of discriminating these coffees from inferior coffees be available.

Coffee cupping quality is a difficult term to define. Each individual tends to have a unique definition. The lack of a uniform definition is irrelevant on an individual household basis, however. In order to increase coffee quality worldwide, a more objective means of defining coffee cupping quality is necessary.

Traditionally, an expert cupper was relied upon to select and evaluate coffees. This afforded a quick and detailed evaluation, but lacked substantial variation in quality analysis as a result of having only one taster. More recently, a trained panel of people has been used in defining and determining cupping quality. A panel is laborious, time consuming and lacks the expert evaluation of coffee. In addition, both methods are biased due to the inherent subjectivity of human tasters. A quantitative, objective measure of quality should measure the same quality characteristics that a panel perceives, but more rapidly, consistently and accurately.

The taste characteristics recognized by a panel are ultimately defined by the chemical composition of the beverage. Therefore, an objective means of defining quality is in terms of coffee chemistry. The chemical composition of the beverage is dependent

upon the genetic composition of the plant, growing conditions, cultural practices and various processing steps.

Green, unroasted coffee is a good choice in the coffee production chain to measure coffee chemistry. This stage is relatively biologically and chemically stable, it is easy to transport and it eliminates several subsequent production steps that can alter coffee chemistry.

This thesis attempts to develop a laboratory method to quantitate specific green coffee chemical components and then determine if they correlate with coffee cupping quality. This will be accomplished by chemically analyzing green coffee, having a trained cupping panel analyze that coffee and then correlate the data using multivariate statistical techniques. Simple organic acids, low molecular weight sugars and chlorogenic acids will be analyzed.

Green coffee samples were harvested from various coffee growing regions in the Hawai'ian islands. Several varieties were picked within a single location. In addition, the same variety was picked from as many of the locations as possible. When possible, samples were collected in two consecutive years. Hybrid coffees from the Hawai'i Agriculture Research Center's (HARC) breeding program were sampled to determine chemical and sensory profiles as well as to explore relationships to the parent plants. Coffees used in this project were planted in 1986-1987 for the Hawai'i State Coffee Trial (Farmer's Bookshelf, 2002). The trial was designed to explore many varieties in different locations in the islands as well as establish plantings to be used for research. The breeding program at HARC began in 1997 and is the source of all Oahu genotypes not part of the state trial (Nagai et al., 2001).

The research for this thesis is part of a joint project at HARC and the University of Hawai'i. I worked in the biochemistry lab at HARC with Mel Jackson developing a method and collecting chemical data for this project. I harvested and processed much of the coffee used herein. The cupping panel and data were organized and collected at the university by Catherine Cavaletto. I assisted in roasting samples the second year. I analyzed all the data with the assistance of Tamara Ticktin of the UH botany department.

Identification of flavor-impacting components could lead to improvements in coffee quality. The genetic and environmental factors that determine the components could be identified and manipulated. The improvement in coffee quality would hopefully lead to higher coffee prices.

The prevention of fraudulent claims might be accomplished by the ability to discriminate coffees by genetic composition, origin of production and method of processing. In addition, this grouping ability would allow for the establishment and control of coffee appellations- a means the wine industry has long used to distinguish and maintain quality. The most efficient technique of discriminating these groups entails using the aforementioned chemical components as the discriminating factors. However, it is also useful to discriminate the groups based on cupping characteristics defined by the panel. This research also includes determining the feasibility of using these data for group discrimination.

CHAPTER 2
SIMULTANEOUS DETERMINATION OF CERTAIN ORGANIC ACIDS AND SUGARS IN GREEN COFFEE

The following is a manuscript prepared for submission to the Journal of Agricultural and Food Chemistry. It describes the development of a method for quantitatively analyzing free sugars and simple organic acids in green coffee. The method took approximately one year to develop and was used to generate some of the data for the multivariate analysis described in later chapters.

Abstract

Development of an ion-exchange HPLC method to simultaneously determine certain organic acids and sugars in green coffee is described. Identifying and quantitating flavor relevant compounds in green coffee offers the potential for an objective measure of cup quality. Presently, a panel of trained experts who rely upon sensory discrimination, a subjective measure, determines coffee quality. Accurate quantitation of individual compounds was verified by single component fortification of green coffee samples and determination of recoveries. Typical recoveries were as follows: malic acid 100.7%, quinic acid 86.7%, citric acid 112.8%, lactic acid 100.0%, sucrose 93.6%, fructose 109.3% and glucose 110.2%. This method is the first to report simultaneous determination of two classes of green coffee components, and provides for a relatively quick and accurate means of determination. Future utilization of this method as a measure of coffee cup quality in green coffee has implications for future coffee breeding, flavor manipulation and genetic transformation.

Introduction

The sensory aspects of coffee quality, also called cup quality, are determined by a panel of trained individuals. These individuals sample an infusion made from roasted coffee and rate it accordingly. Ratings typically include aspects such as acidity, flavor, aroma and body, all of which are determined by the chemical composition of the coffee, the processing methods, the degree of roast and method of brewing.

While this method of quality measurement has been the industry standard, it is a semi-objective measure. A quantitative method of quality determination would save time and resources, eliminate human subjectivity and offer the means to identify components and their concentrations in green bean which could be manipulated to influence quality. Green coffee- dry, unroasted coffee seed with the seed coat removed- is ideal for such investigations as it eliminates some factors that impact quality. There are up to 850 different compounds in roasted coffee compared with approximately 300 in green coffee (Flament, 2002). Many of these compounds are the result of chemical conversion of precursor compounds found in green bean (Clifford, 1985a).

Green coffee is known to contain many unique compounds (Clarke and Macrae, 1985), some of which have already been implicated in the taste characteristics of brewed coffee. For instance, research by Ohiokpehai et al. (1982) suggests that the ratio of the monochlorogenic acids/dichlorogenic acids influences coffee quality. In addition, Rivera (1999) has reported that simple organic acids have discrete, concentration dependent sensory effects. A number of authors have demonstrated that roasting sucrose, either under acidic conditions or in the presence of chlorogenic acids, results in the production of simple organic acids (Crean, 1966; Lukesch, 1956; Nakabayashi, 1978). Nakabayashi

(1978) further noted that the resulting organic acids were identical to those found in roasted coffee infusions. The involvement of reducing sugars in Maillard reactions and the consequent formation of highly volatile aromatic compounds suggests an additional role for sugars in coffee quality (Flament, 2002). The contribution of acids to pH of the coffee brew will also affect sensorial properties due to the dissociation of various organic acid salts and the liberation of highly volatile free acids (Balzer, 2001).

The chlorogenic acids have been studied extensively, thus, they will be ignored here (Ky et al., 1997). Various methods are described for the detection of sugars and organic acids in green coffee (Rogers et al., 1999; Silwar and Lüllmann, 1988; van der Stegen and Duijn, 1987), however, no reported methods exist for the rapid detection of both chemical groups simultaneously. This paper describes a relatively rapid and accurate method for the simultaneous determination of organic acids and sugars in green coffee. It involves one extraction and one separation system, with two detectors linked in series.

Materials and Methods

Materials

Organic acids were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sugars were purchased from Mallinckrodt, Inc. (Pons, KY, USA) All solvents were HPLC grade and were obtained from Burdick and Jackson (Muskegon, MI, USA). All other reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Coffee cherries (*Coffea arabica* var. Typica) were supplied by Kauai Coffee Co., Kauai, Hawai'i. Mature coffee cherries were hand picked, pulped and the mucilage

removed by soaking in water overnight at room temperature followed by a water rinse. The parchment coffee (seed plus seed coat) was dried at 52° C in a forced air oven to a final moisture content of approximately 12% of dry weight, hulled and subsequently stored at -20° C. Immediately prior to analysis and extraction, the green coffee was lypholyzed to complete dryness and then ground to a fine powder using an IKA Universalmühle M20 blade grinder (IKA-Labortechnik, West Germany). Larger particles were removed by passage through a 0.6 mm screen.

Methods

Extraction

One gram of the powdered green coffee was placed in a 125 ml glass beaker and mixed with 10 ml of 8 mM sulfuric acid containing 50% (v/v) methanol. All samples were sonicated for 15 min and filtered through a Whatman GF/A glass fibre filter. Then, a 5 ml aliquot of filtrate was evaporated to a volume of less than 2.5 ml using a rotary evaporator (Tokyo Rikakikai Co., LTD, Japan). Distilled water was added to a final volume of 5 ml. Two mls of the extract were passed through a 1 ml bed volume Sep-Pak® C18 SPE cartridge (Waters), preconditioned with 1 ml of methanol. Residual, unbound extract was eluted by addition of 1 ml of distilled water. The eluants were then filtered through a 0.45 micron nylon filter (Millipore), prior to high performance liquid chromatography (HPLC).

HPLC

HPLC analysis of sugars and organic acids was undertaken using a Shimadzu SCL-10A controller and LC-10AS pump (Shimadzu Corporation, Japan). Chromatographic separations were achieved using a Rezex ROA organic acid ion exchange column (300 x 7.5 mm) with a Carbo-H+ guard column (4 mm x 3 mm), (Phenomenex USA, Torrance, CA). The mobile phase consisted of 6.5 mM sulfuric acid in distilled water at a flow rate of 0.4 ml/min. Twenty microliters of sample were injected. The run time was 24 min followed by a column wash step, using a mobile phase of distilled water at a flow rate of 0.52 ml/min for 10 min. The column was re-equilibrated using the original analytical mobile phase at a flow rate of 0.52 ml/min for a further 27 min. Organic acids were detected using a Shimadzu SPD-10A UV-VIS detector, measuring absorbance at 220 nm (0 .1 AUF). Sugars were detected from the same chromatography run using a Waters 410 differential refractometer (Waters Associates, Inc, USA), connected in series. Plots and peak integrations were performed using a Shimadzu CR501 Chromatopac integrator.

Sample Fortification

Extraction efficiency was determined by fortifying 1 g of dried, ground sample with 5 mg of organic acid, 75 mg sucrose or 3.75 mg of fructose or glucose, and extracting and analyzing as described above. Negative controls, consisting of sample without fortification, were also extracted and analyzed. Each fortification was performed three times.

Quantification

Analytes were quantitated based upon a linear regression equation derived from a standard curve constructed from known concentrations of each analyte. Appropriate standards were chromatographed with each sample set analyzed.

Results and Discussion

Figure 2.1 shows a typical HPLC profile of organic acids in green coffee with UV detection. Peaks were identified by sample fortification with specific organic acids and quantified using standard curves for each corresponding compound. Regression of standard curves for individual acids illustrated strongly linear characteristics, with coefficients of variance (r^2) all greater than 0.99 (figure 2.2). Recoveries obtained from acid fortification are shown in table 2.1. They ranged from 86.7% (+/-3.06%) to 112.8% (+/-5.29%) and demonstrated that the method of extraction was sufficient.

Figure 2.1 Chromatogram from UV Detection.

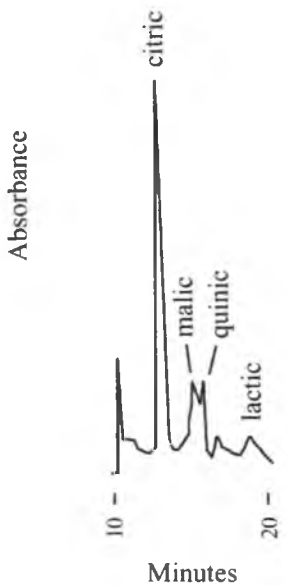
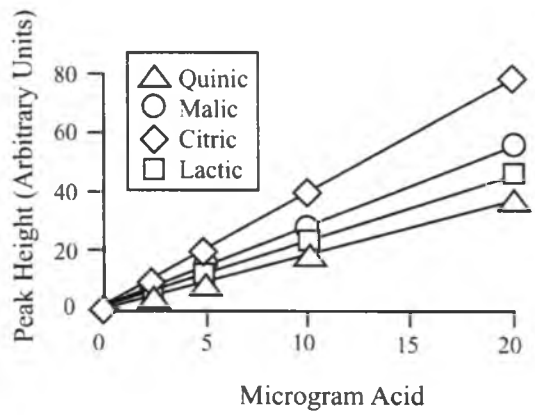


Figure 2.2 Standard Curves for Organic Acids. $r^2 > .99$



Previous reports of organic acid content in arabica green bean were reviewed by Balzer (2001) and gave the following percent on a dry weight basis (% dwb): malic, 0.26-0.67%; citric, 0.5-1.49%; quinic, 0.33-0.61%. The acid content of the coffee analyzed herein has comparable values of 0.41% (malic), 1.37% (citric) and 0.57% (quinic). Maier (1987) found less than 0.1% lactic acid in green arabica coffee. The coffee analyzed here had a similar content of 0.15%.

Additional acids can be detected with this method. Oxalic and pyruvic acids were both identified. On this HPLC column, oxalic acid elutes prior to citric and pyruvic elutes immediately prior to malic. However, recovery from fortified samples was never higher than 70%. Therefore, quantitation of these acids was not undertaken.

Table 2.1. Recoveries, retention times and detection[a] of organic acids and sugars

Compound	Average % Recovery (n=3)	sd[b]	Retention time (min)
Malic acid	100.7	8.1	15.7
Quinic acid	86.7	3.1	16.2
Citric acid	112.8	5.3	13.5
Lactic acid	100	10.6	19.2
Sucrose	93.6	7.2	12.9
Glucose	110.2	6.2	15.3
Fructose	109.3	4.6	16.1

[a]organic acids were detected by UV absorbtion. Sugars were detected using their refractive index
[b]sd=standard deviation

Figure 2.3 shows a typical HPLC profile of sugars and some acids in green coffee detected using differential refraction. Peaks were identified by sample fortification with either sucrose, fructose or glucose and quantified using standard curves for each corresponding compound. Regression of standard curves for individual sugars illustrated linear characteristics, with coefficients of variance (r^2) all greater than 0.99 (figure 2.4).

Recoveries obtained from sugar fortification are shown in table 2.1. They ranged from 93.6% (+/-7.2%) to 110.2% (+/-6.2%) and demonstrated that in addition to the organic acids, the method of extraction described here was also sufficient for sugars, allowing for simultaneous determination.

Figure 2.3 Chromatogram from RI Detection.

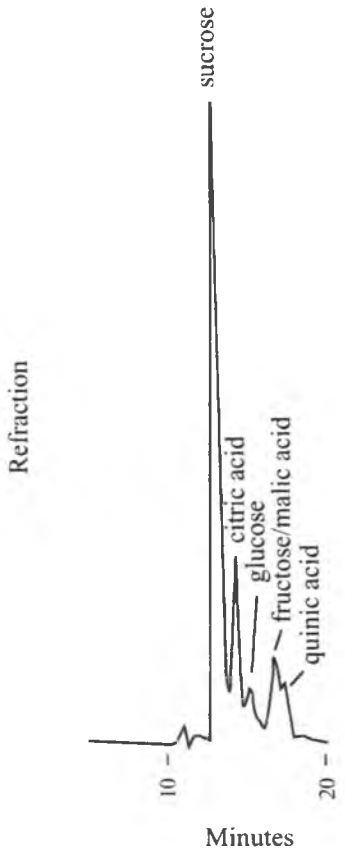
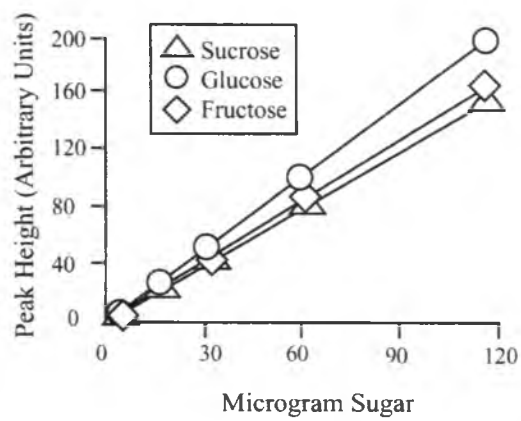


Figure 2.4. Standard Curves for Sugars. $r^2 > .99$



Clifford (1985a) and Trugo (1985) summarize reports that illustrate arabica green bean sugar content (% dwb). Sucrose content ranged from 4.6-8.6%; glucose, 0.03-1.2% and fructose, 0.02-0.3%. Silwar and Lüllmann (1988) demonstrate a greater range for the monosaccharides that extends to 0.45% for glucose and 0.4% for fructose. In general agreement with other literature, the values for sucrose and glucose in the arabica coffee

sample reported here are 7.31 and 0.25 (% dwb), respectively. The amount of fructose in this sample was somewhat higher at 0.56%.

Given the observation that three of the organic acids studied exhibit refractive properties, it was also noted (figure 2.3) that malic acid and fructose co-elute. The additional response from malic acid is the likely cause of the elevated fructose concentration in this sample. While this co-elution prevents accurate quantitation of fructose, the recovery data for this compound is still valid. Malic acid yields a UV and a refractive signal, while fructose only gives a refractive signal. Therefore, the contribution of malic acid to the apparent fructose signal can be accounted for. This results in a corrected fructose concentration of 0.24% dwb.

This method can be used to test the hypothesis that certain chemical precursors can be used to evaluate brewed coffee quality. Rivera (1999) showed that addition of 10.8 ppm of citric acid to brewed coffee imparts a mildly acidic "apple-like" quality when cupped by an expert panel. Similarly, he found that malic acid, added at 20 ppm to brewed coffee, imparted a "bright, sweet, orange" taste. These acids are naturally present in the roasted, and consequently, brewed coffee (Balzer, 2001). It may therefore be possible to predict the sensorial properties of brewed coffee by quantitation of organic acids and sugars in green bean. The elucidation of such a hypothesis has far-reaching implications for the coffee industry. A greater understanding of the importance of flavor precursors in green coffee might create possibilities for flavor manipulation. The ability to quickly and easily determine green coffee chemical profiles suggests research avenues in coffee breeding and genetic transformation that would permit rapid development of varieties with improved cup quality.

CHAPTER 3
MATERIALS, METHODS AND STATISTICAL ANALYSIS

Coffee materials

Coffee samples were harvested in the fall of 2000 and 2001. Ripe cherries were hand-picked, pulped and fermented overnight. The year 2000 coffee samples were oven-dried at 52° C and the 2001 samples were dried outdoors. All coffees were dried to a moisture content of approximately 12%.

Coffee varieties were picked from numerous locations in Hawai'i, U.S.A. These locations were: Eleele, Kauai (91); Waiakea, Hawai'i (122); Kainaliu, Hawai'i (701); Lahaina, Maui (290); and Kunia, Oahu (87). Numbers in parentheses are meters above sea level. Latitudes ranged from 19° - 22° N and longitudes ranged from 155° - 160° W. All the farms were irrigated and fertilized when necessary. All coffee was grown in full sun. The following varieties were harvested from these locations: Catimor, Mokka, Blue Mountain x Mokka hybrid (BMxM), Red Catuai x Mokka hybrid (CIxM), Red Catuai, Red Caturra, Yellow Catuai, Yellow Caturra and Typica. Coffees were either part of the Hawai'i State Coffee Trial (Farmer's Bookshelf, 2002) or the HARC breeding program (Nagai et al., 2001).

All coffee was sorted by size and defects and peaberries were removed. The green coffee was stored at -20° C until needed for chemical analysis and cupping. Each sample was split into an aliquot for the cupping and an aliquot for the chemistry. When possible, the same size coffee bean was analyzed for chemical and cupping characteristics.

Method for cupping

A trained panel at the University of Hawai'i at Mānoa cupped the coffee. Coffee was roasted the day prior to cupping. Seventy grams of green bean were roasted in a Probat PRE-1 lab roaster at 200° C to an L level of approximately 18 on the LAB scale. Color was measured with a Hunterlab Colorimeter, model D25M-9 (Hunterlab Associates Laboratories, Inc., Reston, VA). The coffee was ground using a Santos grinder to an "express" size.

Each panelist received 7.25 g of coffee presented in a ceramic bouillon cup for rating of the dry grounds aroma. Immediately following, 150 ml of water at a temperature just below boiling was added. Two minutes after water was added, wet aroma was assessed. Five minutes after the addition of water, acidity, flavor and body were evaluated. Seven panelists cupped the coffee in individual tasting booths. Coffees were grouped by variety for the cupping and the test was replicated 3 times. Coffees were rated on a 0 (not present) to 5 (intense) scale.

Method for organic acid analysis

Simple organic acids and sugars were analyzed as described in chapter 2: materials and methods. The chlorogenic acids were extracted and analyzed as follows.

Materials for chlorogenic acid analysis

All solvents were purchased from Burdick and Jackson (Muskegon, MI, USA) and were HPLC grade. 5-caffeoylquinic acid (5-CQA), triflouroacetic acid (TFA) and

potassium ferrocyanide were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Zinc acetate was obtained from Mallinckrodt, Inc. (Pons, KY, USA). All other reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

High Performance Liquid Chromatography (HPLC) analysis of chlorogenic acids was undertaken using a Shimadzu SCL-10A controller and LC-10AS pump (Shimadzu Corporation, Japan). Chromatographic separations were achieved using a YMC Basic S-5 column (4.6 x 250 mm)(Waters Corp., Millford, MA, USA). Detection was achieved using a Shimadzu SPD-10A UV-VIS detector, measuring absorbance at 315 nm (0 .1 AUF). Plots and peak integrations were performed using a Shimadzu CR501 Chromatopac integrator.

Method for chlorogenic acid analysis

Chlorogenic acids were analyzed by a method adapted from Balyaya and Clifford (1995). Immediately prior to analysis and extraction, the green coffee was lypholyzed to complete dryness and then ground to a fine powder using an IKA Universalmühle M20 blade grinder (IKA-Labortechnik, West Germany). Larger particles were removed by passage through a 0.589 mm screen.

One half gram of coffee was placed in a round-bottom flask and mixed with 100 ml of 70% methanol (v/v). The sample was refluxed for 1 hr. After cooling to room temperature, 1 ml each of Carrez reagents was added (Carrez 1 = 21.9 g zinc acetate dihydrate and 3 ml glacial acetic acid in 100 ml H_2O; Carrez 2 = 10.6g potassium ferrocyanide trihydrate in 100 ml H_2O) with stirring after each addition. Once the

precipitate settled, samples were filtered using a .45 micron nylon filter (Millipore) and prepared for HPLC.

The mobile phase consisted of solution A: .06% TFA and solution B: .06% TFA in 45% acetonitrile (v/v). Prior to sample injection, the mobile phase used 20% of solution B and increased to 45% in 26 min. The concentration of solution B then increased to 100% in the next 10 min. The column was then allowed to equilibrate at 20% solution B for 4 min. Twenty microliters of each sample were injected at a flow rate of 1 ml/min. The chlorogenic acids were detected at 315 nm (.1 AUFS).

Seven chlorogenic acids were identified from their relative retention times and molar absorbencies (Balyaya and Clifford, 1995; Clifford et al., 1985). Quantification was based upon a linear regression equation derived from a standard curve of known concentrations of 5-CQA. An appropriate standard was chromatographed with each sample set analyzed. All extractions were repeated in triplicate.

Statistical analysis

The raw data and explanations of the variables can be found in the appendix. These data were used for statistical analysis.

Multivariate statistical analysis was performed using SAS statistical software, version 8.02 (SAS Institute Inc., Cary, N.C.). Exploratory analysis of the data was performed using principal components analysis (PCA). Discriminate analysis (DA) was used to examine group differences identified from the PCA. The relationship between green coffee chemistry and cupping characteristics of cupped coffee was illuminated using canonical correlation analysis (CANCORR).

Multivariate statistics convert a large number of variables into linear combinations of fewer variables through the use of matrix algebra. These linear combinations are (eigen)vectors that are gradients of variation. Generally, the first 2 or 3 vectors capture most (>70%) of the variation in the data set. The type of variation captured is dependent upon the specific technique used. For example, principal component analysis, an ordination technique, combines variables into components and maximizes the variation within each component. While PCA aims to maximize variation within a single set of variables, CANCORR strives to maximize the correlation between two sets of linearly combined variables. Discriminate analysis also creates gradients of variation, however, it maximizes that variation among groups and minimizes it within groups.

Like univariate statistics, a large sample size is important for good interpretation of patterns in a data set. In addition, multivariate statistics require a large sample to variable ratio. As the ratio becomes smaller, the usefulness of the statistic decreases and interpretation should be made with caution.

The PCA was run twice. The first analysis used the raw chemical data and algebraic combinations of the raw data (see appendix). The second analysis used the cupping data. Prior to using DA, the data were treated to a stepwise analysis to eliminate multicollinearity. The variables selected from the stepwise analysis depended on the grouping in question. The groups and variables will be discussed in the results section of chapter 5. Reduction of multicollinearity for CANCORR was accomplished by removing variables with pairwise correlations of .7 or higher. The remaining chemical variables were quinic acid, malic acid, citric acid, lactic acid, sucrose, 3-CQA, 4,5-diCQA and the

monoCQA/diCQA molar ratio. No multicollinearity existed for the cupping data, thus, all 5 variables were used.

CHAPTER 4
CORRELATION OF GREEN COFFEE CHEMISTRY WITH CUPPING QUALITY

Introduction

The quality of brewed coffee is influenced by many factors: genetics, cultural practices, environmental conditions, processing, storage, roasting and brewing. This quality is defined and described by trained individuals. These individuals ultimately are describing sensory responses that are determined by the chemical composition of the beverage.

Flavor chemists have spent many years exploring roasted and brewed coffee in attempts to identify the most important flavor compounds. The search has revealed over 800 unique compounds, all of which are derived from precursors found in unroasted coffee (Flament, 2002). Identifying relevant precursors in green coffee simplifies quality research by eliminating changes in the coffee due to roasting and the limitations of human tasters (Feria-Morales, 2002).

Of the two commercially important coffee species, *Coffea arabica* and *C. canephora*, it is generally accepted that *C. arabica* is of higher quality. Major chemical differences exist in the green beans of these species. Examining these differences may lead to a better understanding of the chemistry behind coffee quality.

Arabica coffee contains about twice as many low molecular weight (lmw) sugars (Silwar and Lüllmann, 1988) and significantly less chlorogenic acids (CGA) than *C. canephora* (Clifford, 1985b). While the content of short-chained organic acids in these species is similar, their contribution to pH of the coffee brew will affect sensory properties due to the dissociation of various organic acid salts and the liberation of highly

volatile free acids (Balzer, 2001). In addition, Rivera (1999) reported that simple organic acids have discrete, concentration dependent sensory effects. Similarly, Maier (1987) demonstrated the importance of citric and malic acids to the acid taste in coffee. As coffee beans mature, a change in content of organic acids and sugars is observed (Rogers et al., 1999; Mazzafera, 1999)). This may be relevant to discussions of quality as immature coffee beans are generally recognized as affecting coffee quality negatively.

A number of authors have demonstrated that roasting sucrose, either under acidic conditions or in the presence of chlorogenic acids, results in the production of simple organic acids (Crean, 1966; Lukesch, 1956; Nakabayashi, 1978). Nakabayashi (1978) further noted that the resulting organic acids were identical to those found in roasted coffee infusions. Ginz et al. (2000) demonstrated the role of lmw sugars as acid precursors by infusing green coffee with these compounds and then roasting it. The involvement of reducing sugars in Maillard reactions and the consequent formation of highly volatile aromatic compounds suggests an additional role for sugars in coffee quality (Flament, 2002). Chagas (1994) observed a positive relationship between beverage quality and reducing and non-reducing sugar content in green beans.

Researchers have been considering the role of chlorogenic acids in coffee quality for many years. The CGAs are a complex group of chemicals consisting of quinic acid esters, each with multiple isomers. The most numerous and most frequently studied of the CGAs are the caffeoylquinic acids (CQA) (Clifford, 1985b). Amorim et al. (1974b) showed that an increase in the total CGA content of green bean depresses beverage quality. The molar ratio of the monocaffeoylquinic acids (mCQA) to the dicaffeoylquinic acids (diCQA) has been implicated in coffee quality, with a lower ratio

signifying poorer coffee (Ohiokpehai et al., 1982). As coffee beans mature, a significant change in the ratio can be observed (Clifford et al., 1987; De Menezes, 1994). Inclusion of immature beans in a brew will likely depress the quality due to the lowering of this ratio (De Menezes, 1994). Bicci et al. (1995) and Moreira et al. (2001) demonstrated that coffees of different origins or varieties had cupping profiles that differed in a similar fashion to their chlorogenic acid profile. However, neither study reported a statistical correlation; they only compared the chemical and cupping data.

The aim of this research is to correlate green coffee chemistry to cupping quality.

Materials and Methods

For materials and methods, refer to chapter 3.

Results

Canonical correlation analysis did not elucidate a strong correlation between green coffee chemistry and cupping. The correlations of variate pairs yielded a promising relationship. However, the redundancy analysis showed that little variation was being captured in the chemical variates.

The variables are linearly combined into eigenvectors, also known as canonical variates. The squared canonical correlation represents the correlation between pairs of variates. Each pair consists of one variate derived from the chemical variables and one derived from the cupping variables. Redundancy analysis measures the variation in one set of variables that is explained by the variates of the other set of variables.

Only two eigenvector pairs were statistically significant at the .08 level. The squared canonical correlations were .578 and .457. Thus, the first chemical variate accounted for 58% of the variation in the first cupping variate (and vice versa) and the second chemical variate accounted for 46% of the variation in the second cupping variate (and vice versa).

The standardized variance of the cupping variables explained by the first two chemical variates (redundancy) was .177 and .06. Thus, these two chemistry variates only account for 18% and 6% of the variation in the cupping variables, respectively.

Discussion

Previous work on correlating green coffee chemistry and cupping quality has focused on the chlorogenic acids. While some evidence suggests that they play a role in cupping quality, no definitive connection has ever been established. This study was unable to confirm a direct correlation between the CGAs and cupping quality. Furthermore, the analysis of simple organic acids and sugars did not reveal any correlation to quality. This supports a report by Amorim et al. (1974a) that showed no correlation between coffee quality and ethanol soluble sugars or reducing sugars.

Although no correlation was found in this study, there is still hope that a correlation might be found. It is possible that the selected chemicals determine cupping characteristics not rated herein. Conversely, different chemicals may determine the selected cupping characteristics. Therefore, the selection of chemicals and cupping characteristics may be appropriate, however, this study simply failed to pick the correct combination.

The impact of roasting cannot be ignored. A multitude of chemical changes occur during the roasting process. The alteration caused by this process may prevent any direct correlation from being established. This study presumes that the changes do so in a quantitative manner that is measurable from green bean to cup. However, no single chemical must directly translate into any easily measurable cupping characteristic.

Future avenues of research must be two-fold. First, the absolute number of chemicals analyzed and the number of chemical classes must be increased. In addition, more cupping characteristics should be examined.

Second, to eliminate the changes caused by roasting, this study should be repeated, but with roasted coffee instead of green coffee. If chemicals in roasted coffee could be identified as major contributors to cupping quality, chemists could attempt to determine the green coffee precursors of those specific chemicals.

CHAPTER 5
GROUP DISCRIMINATION

Introduction

The ability to distinguish coffee by an objective means has long been sought after by the coffee industry. Coffees that can be discriminated by origin, genetic profile or processing method would be difficult to adulterate or intentionally mislabel. Furthermore, discrimination of coffee groups could assist in creating a system of coffee appellations by serving as a monitoring and verification tool.

Many researchers have concentrated on distinguishing the two commercially important coffee species, *Coffea arabica* and *C. canephora* and their hybrids (Anderson and Smith, 2002; Briandent, et al., 1996; Downey et al., 1997; Guerrero et al., 2001; Kemsly et al., 1995). Many characteristics using various detection methods were used to distinguish these roasted coffees.

Clifford and Jarvis (1988) observed differences in chlorogenic acids in 42 samples of unroasted *C. canephora* from many locations. The chlorogenic acids were used as taxonomic criteria for species in the tribe Coffeae (Clifford et al., 1989). Though the most distinguishing profiles occurred in commercially unimportant species, the noted differences suggest that chemical profiles may contain group discriminating data. Bicchi et al. (1995) used CGAs and PCA to distinguish arabica and robusta green and roasted coffees by origin. The study was even able to distinguish. one species, in one origin, from different plantations. The researchers did not report the use of a discriminating analysis.

This research aims to use simple organic acids, sucrose and the chlorogenic acids to discriminate coffees by locations within an origin, varieties of *C. arabica*, and a processing method (forced-air/oven or sun drying of parchment coffee).

Materials and Methods

For materials and methods, refer to chapter 3.

Results

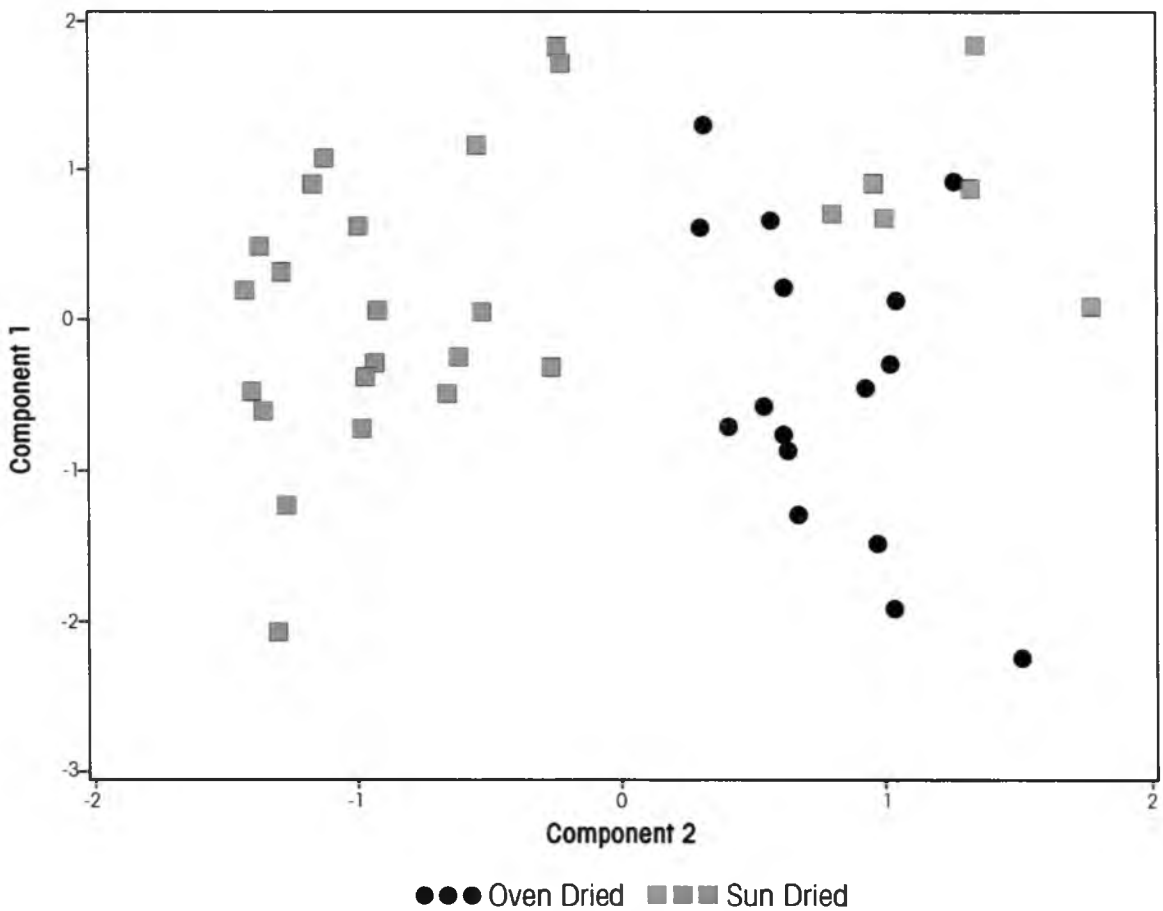
The results from the PCA showed clusters using the chemical variables and the cupping variables separately. Forty-three samples were used in the analysis. Both sets of variables showed clusters of varieties, locations and drying method. Figure 5.1 shows a biplot of the first 2 principal components using the cupping data plotted by drying method. The six sun-dried samples clustered with the oven-dried samples are all of the variety Red Catuai. In fact, all but one Red Catuai sample mapped on the high end of component 2. Component 1 is positively associated with dry aroma, wet aroma and flavor. Acidity and body are positively associated with component 2, which actually distinguishes these groups. Thus, higher values on the components are represented by higher values of the cupping variables.

Although the drying method sharply divides the samples, groupings by variety exist. Figure 5.2 is also a biplot of the first 2 components of the cupping analysis, but mapped by variety. BMxM, Catimor and Typica group on the aroma/flavor component. Yellow Caturra, Catimor , Typica, Mokka, BMxM and CIxM align on the acidity/body

component. All but one sample of the Red Catuai variety congregates on both components 1 and 2. This outlier is the only one from Kona and oven-dried.

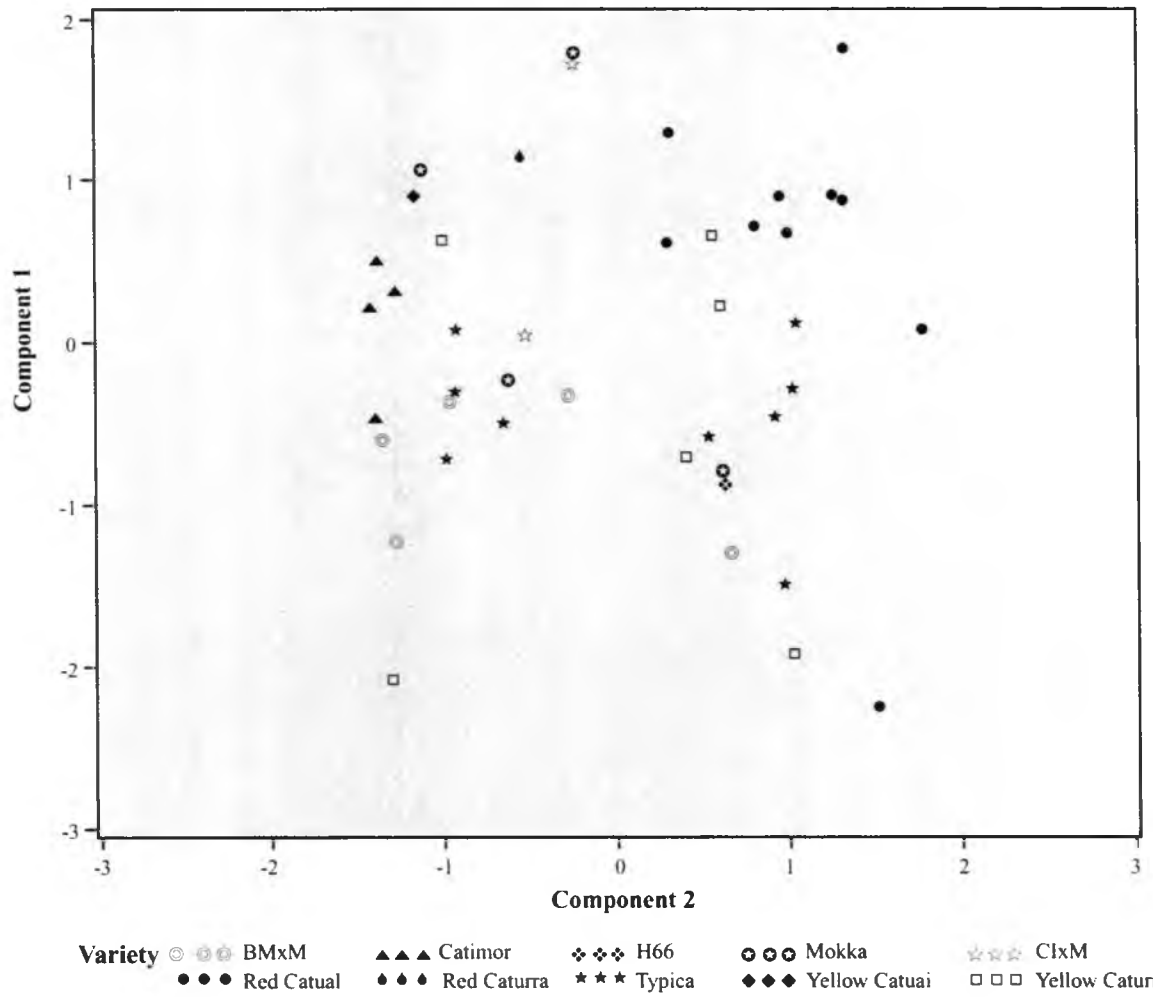
The distinct separation of samples by drying method tended to overshadow groupings by location. However, some groupings could be identified; Kauai and Kona grouped on components 1 and 2 (graph not shown). The Kona groups were distinguished by being low on the aroma/flavor component. One Kona sample outlier, a sun-dried Red Catuai, mapped high on the flavor/aroma component The Kauai group was grouped near 0 on both axes. The other locations did not group well by cupping attributes.

Figure 5.1. Biplot of the first 2 principal components of the cupping analysis plotted by drying method



When the same analysis was plotted by variety, several groupings appeared. The Mokka, Mokka hybrids and Yellow Caturra grouped on the CGA component. Mokka grouped on component 3 and BMxM grouped on component 2. Catimor loosely grouped on all 3 components. Component 2 was represented by malic acid, citric acid and total acid. When drying method was mapped on the first 2 components, two distinct groups could be seen.

Figure 5.2. Biplot of the first 2 principal components of the cupping analysis plotted by variety



The resolution of groups prompted the use of discriminate analysis to further understand both the nature of the groups and the ability of the data to define the groups. Essentially, this analysis creates canonical functions that maximize between group differences and minimizes within group differences. The analysis produces n-1 canonical variates where n is the number of groups. Each sample is sorted into the group to which its distance from the group mean is smallest. Results are presented in a table showing the known group of each sample, the group the sample was placed into and the percent error of classification. Testing the validity of the sorting is done with the jackknife resampling procedure. This verifies that the groups are true groups and not functions of the particular data set.

Chemical variables for group discrimination were chosen based on a stepwise analysis. Since this analysis does not always produce the variables that are best able to discriminate groups, other variables were sometimes added to improve discrimination. Chemical variables were separated from cupping variables for the stepwise analysis and group discrimination.

A total of 43 samples were analyzed in this study. However, at least 3 samples must exist for each group to run discriminate analysis. Appropriate samples were eliminated from each analysis to fulfill this requirement.

Forty-three samples were used to discriminate oven drying (16 samples) from sun drying (27 samples). Oven drying correlated positively with the single canonical variate and sun drying correlated negatively. Therefore, any variable that correlates positively with the variate has larger values in the oven-dried group. Table 5.1 shows the variables

used to classify the groups, the percent accuracy of the classification and the percent classification accuracy from the jackknife procedure.

Table 5.1. Discrimination of coffee by drying method

Variables used in classification	Percent correct classification	Percent correct classification from jackknife procedure
Lactic acid (+)	92.5	86
Lactic acid (+), 3-CQA (-)	94.5	94.5
Dry aroma (-), wet aroma (-) acidity (+) flavor (-), body (+)	100	97
Acidity (+)	85	85
Acidity (+), body (+)	98	95

Variables that correlate positively (+) or negatively (-) with the canonical variate

Forty-one samples were used to discriminate coffee by location that included samples from both drying methods. Four locations were represented: Kauai (3 samples), Kona (6 samples), Kunia (25 samples) and Maui (7 samples). Table 5.2 shows the variables used to classify the groups, the percent accuracy of the classification and the percent classification accuracy from the jackknife procedure. Due to the use of several variates, identifying the precise relationship between the variables and the groups is difficult. Therefore, no report is given for any other correlations like the ones found in Table 5.1.

Table 5.2. Discrimination of coffee by location including samples from both drying methods

Variables used in classification	Percent correct classification	Percent correct classification from jackknife procedure
Quinic acid, sucrose, total CQA, molar ratio	87	33
Dry aroma, wet aroma, acidity, flavor, body	89	13

Fourteen samples were used to discriminate coffee by location that included only oven-dried samples. Four locations were represented: Kauai (3 samples), Kona (3 samples), Kunia (4 samples) and Maui (4 samples). Table 5.3 shows the variables used to classify the groups, the percent accuracy of the classification and the percent classification accuracy from the jackknife procedure.

Table 5.3. Discrimination of coffee by location including only oven-dried samples

Variables used in classification	Percent correct classification	Percent correct classification from jackknife procedure
Quinic acid, 3,4-diCQA, 3,5-diCQA, 5-FQA, molar ratio	100	94
Dry aroma, wet aroma, acidity, flavor, body	79	23

Twenty-seven samples were used to discriminate coffee by location that included only sun-dried samples. Three locations were represented: Kona (3 samples), Kunia (21 samples) and Maui (3 samples). Table 5.4 shows the variables used to classify the groups, the percent accuracy of the classification and the percent classification accuracy from the jackknife procedure.

Table 5.4. Discrimination of coffee by location including only sun-dried samples

Variables used in classification	Percent correct classification	Percent correct classification from jackknife procedure
Lactic acid, 3,5-diCQA, total diCQA	100	34
Dry aroma, wet aroma, acidity, flavor, body	73	40

Thirty-eight samples of both drying methods were used to discriminate coffee by variety. Six varieties were represented: BMxM (5 samples), Catimor (4 samples), Mokka

(4 samples), Red Cataui (10 samples), Typica (9 samples) and Yellow Caturra (6 samples). Table 5.5 shows the variables used to classify the groups, the percent accuracy of the classification and the percent classification accuracy from the jackknife procedure.

Table 5.5. Discrimination of coffee by variety including samples from both drying methods

Variables used in classification	Percent correct classification	Percent correct classification from jackknife procedure
Quinic acid, malic acid, 5-FQA, total diCQA	92	15
Dry aroma, wet aroma, acidity, flavor, body	100	28

Thirteen samples were used to discriminate coffee by variety including only oven-dried samples. Three varieties were represented: Red Catuai (4 samples), Typica (5 samples) and Yellow Caturra (4 samples). Table 5.6 shows the variables used to classify the groups, the percent accuracy of the classification and the percent classification accuracy from the jackknife procedure.

Table 5.6. Discrimination of coffee by variety including only oven-dried samples

Variables used in classification	Percent correct classification	Percent correct classification from jackknife procedure
Sucrose, total CQA	85	85
Dry aroma, wet aroma, acidity, flavor, body	100	47

Twenty-one samples were used to discriminate coffee by variety including only sun-dried samples. Five varieties were represented: BMxM (4 samples), Catimor (4 samples), Mokka (3 samples), Red Catuai (6 samples), and Typica (4 samples). Table 5.7 shows the variables used to classify the groups, the percent accuracy of the classification and the percent classification accuracy from the jackknife procedure.

Table 5.7. Discrimination of coffee by variety including only sun-dried samples

Variables used in classification	Percent correct classification	Percent correct classification from jackknife procedure
Quinic acid, malic acid, lactic acid	97	17
Dry aroma, wet aroma, acidity, flavor, body	100	20

Discussion

Principal Component Analysis

The PCA analysis produced groups in every analysis. The drying method had the most dominant effect on both the chemistry and cupping of the coffee. Oven-dried coffees were higher in acidity and body than sun-dried coffees. The only exception seems to be Red Catuai since its samples group together in the upper right corner of the graph. Drying does not appear to affect aroma or coffee flavor intensity. Locations and varieties, although split along the second component, always fall along the same general spot on the first component.

The varietal groupings discovered from the cupping analysis are not unexpected; different varieties are known to taste differently. An intriguing result is that the taste of coffee seems to be influenced more by genetic composition (variety) than environmental conditions (location). This is not only supported by the presence of distinct groupings by variety, but also by the fewer numbers of groups found when mapped by location.

Interpretation of cupping characteristics of varieties and locations must be made with caution because of the influence of drying method. Kona grown varieties seem to have relatively less dry aroma, wet aroma and flavor than varieties from other locations whereas Kauai grown varieties appear to be average on all attributes.

Most variety groupings appeared around 0 on the first component. Red Catuai fell high on this axis, consequently, it has higher values for aroma and flavor. The single Red Catuai outlier may suggest that variety is not always dominant over location. Concurrently, the Kona Red Catuai suggests that location is not dominant over variety. It seems that variety, location and drying method all interact to determine the final taste characteristics of some coffees. Overall, Red Catuai can be described as being greater or more intense in the cupping attributes than other varieties studied.

Mokka and the Mokka hybrids all align on the second component, though in slightly different locations. It is possible that the genetic characteristics of Mokka that influences body and acidity have little variation. Unfortunately, whether this response is true of all varieties cannot be determined here.

The role of 3,5-diCQA in group separation has been reported by Moreira et al. (2001). This research supports the idea that CGAs can be used to describe coffees by locations as well as varieties. However, this analysis was unable to more clearly define the roles of those CGAs.

The Kona grown varieties divided into definite groups when analyzed with the chemical data and cupping data. While the CANCORR analysis did not find a direct link between chemistry and cupping, this information suggests that a connection does in fact exist. Further research should attempt to elucidate this relationship.

Discriminate Analysis

The effect of drying method is also seen when using DA. Oven-dried coffees contain more lactic acid and taste more acidy than sun-dried coffees. Lactic acid is the

most significant chemical difference as it can discriminate the groups extremely well by itself. The addition of 3-CQA only slightly improves the classification rate. For a practical application, simply analyzing lactic acid would be better as it would require only one chemical extraction and analysis.

The data also show distinct sensory differences in the samples. Perfect group discrimination is achieved using all 5 cupping variables. Most of the discriminating power, however, is due to acidity. Although lactic acid and acidity are greater in magnitude in oven-dried coffee, no correlation can be made between the two variables. When lactic acid is linearly regressed against acidity, the r^2 value is equal to .24. Large concentrations of lactic acid in green coffee do not necessarily mean greater acidity in brewed coffee.

The initial classification of samples into locations was very good. However, the resampling results were poor with both variable sets. Low resampling classification suggests that these groups do not separate very well using these characteristics. Rather, the separation is due to the particular nature of this sample set.

The effects of drying method were very strong. Therefore, discriminations were carried out separately on the oven-dried and sun-dried samples. Dual analyses were also done for group discrimination by variety.

The chemistry of the oven-dried samples is distinct among the four locations as shown by the high classification rates. It is important to note that all the chemical variables used in the separation are chlorogenic acids or components of these acids. This is in agreement with work that used CGAs for group discrimination (Bicchi et al., 1995). Unlike the drying method data, the cupping data for this discrimination was unable to

clearly separate groups. This supports the idea that location plays a minor role in coffee cupping quality. However, this conclusion would be premature because cultural practices and environmental conditions were not controlled rigorously. Even though separation by chemistry was successful, it is possible that the small group sizes (<5 in each group) were insufficient for discriminate analysis using the cupping data.

The sun-dried samples initially separated into their respective groups extremely well based on the chemical variables and moderately well using the cupping variables. Neither jackknife analysis showed true group separations. The ease of group discrimination of the oven-dried samples suggests that the sun-dried samples also would discriminate easily. The most likely explanation for the poor discrimination is the unequal group sizes. The Maui and Kona groups only had 3 samples each. Unbalanced group sizes often yield poor results because samples are more likely to be randomly placed in a large group (McGarigal et al., 2000).

Not surprisingly, good group discrimination by variety was not achieved using samples from both drying methods. However, the oven-dried samples were discriminated by variety using just sucrose and total CQAs. Separation of groups using the cupping variables was unsuccessful.

Group discrimination by variety of the sun-dried samples did not occur using the chemistry variables. Many combinations of variables were used in the analysis and all produced low classification rates from the jackknife procedure. It is interesting to note that when samples were misclassified, they were nearly always mislabeled as Red Catuai. The peculiar nature of this variety was also seen in figure 5.1 under different conditions. The cupping variables are unable to discriminate true varietal groups.

Group discrimination of coffee using simple organic acids, sucrose and chlorogenic acids is possible. Every discriminate analysis yielded a moderate to excellent initial group classification. Even though many of these were not supported by strong jackknife classifications, their importance as preliminary data should not be ignored. The total sample size and group sizes were too small for these multivariate analyses. If the study were repeated with many more samples, the jackknife classification may have been as high as the initial classification (Ticktin, personal communication).

CHAPTER 6
CONCLUSION

This research successfully developed a method to analyze green coffee for simple organic acids and low molecular weight sugars simultaneously. No correlation could be established between green coffee chemistry and cupping quality. The chemical and cupping variables were able to distinguish coffee by drying method, location and variety.

Using individual characteristics, whether derived from coffee chemistry or cupping, is not useful for describing coffee. Coffee is too complex to be accurately defined and understood by isolated pieces of information. Coffee profiles, generated from combinations of chemicals, cupping characteristics or both must be used to analyze and understand coffee quality and the response of coffee to genetics and the environment

One objective of this study was to distinguish coffee groups and predict group membership (discriminate) using DA. Both the PCA and DA proved useful in distinguishing and defining groups. However, predicting group membership was more elusive. If more samples had been analyzed and group sizes were larger, the outcome of the discrimination may have been more definitive.

The effects of drying method on cupping quality and chemistry were very pronounced. Discrimination of these groups was very successful. Coffees dried by forced air tasted more acidic and contained more lactic acid than coffees dried in the sun.

Coffee profiles were used to understand the relationship between parents and offspring. The Mokka hybrids had similarities in sensory characteristics and chlorogenic acids. Unfortunately, further description could not be accomplished. Nonetheless,

researchers could use this type of data to improve quality through traditional breeding methods or modern molecular techniques.

A shortcoming of this study was the small sample size; it affected most of the data analysis. Parent-offspring relationships could be elucidated further given a larger number of these pairings. In addition, the relationship would be understood best when offspring of known parent plants were analyzed together.

Probably, a larger sample size would improve the results of the discrimination and, perhaps, the correlation analysis. The benefits of group discrimination to researchers and the coffee industry are great. A better understanding of environmental and genetic effects on coffee quality would unfold, creating new or improved practices for quality manipulation. Reliable discrimination would also allow for fraud protection and the use of appellations.

Several possibilities may explain why no correlation was found between green coffee chemistry and cupping quality. The chemicals selected for correlation may contribute to cupping attributes not evaluated here. Conversely, the chemicals that correlate to the selected cupping characteristics may not have been analyzed. Finally, the roasting process may alter coffee chemistry to an extent that prevents correlation.

These data begin to show how coffee chemistry defines coffee quality and the plant's response to genetics and environment. Expansion of this understanding requires studying more chemical compounds in green coffee. Adding more chemicals to a coffee profile will improve the power and versatility of the profile. Likewise, augmenting the cupping profile would be beneficial.

The data produced here demonstrate that coffee profiling can discriminate coffee. Analysis of single attributes cannot. Future research that is designed to apprehend coffee groups and cupping quality must include profiling.

APPENDIX
RAW DATA

	Variety	Location	Drying	Quinic	Malic	Citric	Lactic	Total Acid	Sucrose
1	BMxM	Kunia	oven-dried	0.54	0.71	1.41	0.15	2.81	7.88
2	BMxM	Kunia	sun-dried	0.35	0.55	1.28	0.12	2.30	6.95
3	BMxM	Kunia	sun-dried	0.43	0.50	1.04	0.15	2.12	6.59
4	BMxM	Kunia	sun-dried	0.36	0.56	1.26	0.11	2.28	6.73
5	BMxM	Kunia	sun-dried	0.36	0.56	1.41	0.11	2.43	6.78
6	Catimor	Kunia	sun-dried	0.61	0.56	1.45	0.15	2.76	8.44
7	Catimor	Kunia	sun-dried	0.59	0.55	1.31	0.11	2.56	7.78
8	Catimor	Kunia	sun-dried	0.60	0.49	1.43	0.13	2.65	7.92
9	Catimor	Kunia	sun-dried	0.56	0.43	1.19	0.09	2.26	6.99
10	H66	Kunia	oven-dried	0.41	0.66	1.21	0.19	2.47	7.34
11	Mokka	Maui	oven-dried	0.59	0.58	1.23	0.19	2.58	7.41
12	Mokka	Kunia	sun-dried	0.48	0.41	1.12	0.16	2.18	6.85
13	Mokka	Kunia	sun-dried	0.45	0.48	1.17	0.08	2.18	6.15
14	Mokka	Kunia	sun-dried	0.50	0.43	1.03	0.13	2.08	7.07
15	CIxM	Kunia	sun-dried	0.44	0.41	1.21	0.07	2.12	6.96
16	CIxM	Kunia	sun-dried	0.57	0.44	1.07	0.10	2.18	6.74
17	Red Catuai	Hilo	oven-dried	0.55	0.40	1.21	0.16	2.32	10.39
18	Red Catuai	Kauai	oven-dried	0.70	0.57	1.30	0.17	2.74	7.35
19	Red Catuai	Kona	oven-dried	0.58	0.41	0.99	0.18	2.16	11.19
20	Red Catuai	Maui	oven-dried	0.70	0.68	1.24	0.21	2.83	8.53
21	Red Catuai	Maui	sun-dried	0.50	0.48	1.29	0.12	2.38	6.31
22	Red Catuai	Kona	sun-dried	0.45	0.41	0.90	0.00	1.76	6.51
23	Red Catuai	Kunia	sun-dried	0.52	0.44	1.14	0.09	2.19	7.73
24	Red Catuai	Kunia	sun-dried	0.42	0.28	1.10	0.12	1.91	5.39
25	Red Catuai	Kunia	sun-dried	0.55	0.52	1.34	0.14	2.56	9.12
26	Red Catuai	Kunia	sun-dried	0.47	0.38	1.20	0.11	2.17	6.43
27	Red Caturra	Kunia	sun-dried	0.58	0.54	1.50	0.13	2.76	7.67
28	Typica	Hilo	oven-dried	0.57	0.40	1.36	0.18	2.50	10.48
29	Typica	Kauai	oven-dried	0.57	0.41	1.41	0.15	2.55	8.27
30	Typica	Kona	oven-dried	0.55	0.34	0.70	0.14	1.74	9.25
31	Typica	Kunia	oven-dried	0.44	0.51	1.28	0.18	2.41	7.20
32	Typica	Maui	oven-dried	0.78	0.55	1.80	0.15	3.27	8.33
33	Typica	Maui	sun-dried	0.55	0.57	1.39	0.10	2.60	6.13
34	Typica	Kona	sun-dried	0.54	0.52	1.01	0.10	2.18	7.36
35	Typica	Kunia	sun-dried	0.55	0.45	1.34	0.07	2.41	6.16
36	Typica	Kunia	sun-dried	0.53	0.49	1.47	0.09	2.59	7.97
37	Yellow Catuai	Kunia	sun-dried	0.56	0.47	1.40	0.07	2.50	6.78
38	Yellow Caturra	Kauai	oven-dried	0.77	0.57	1.38	0.17	2.88	6.91
39	Yellow Caturra	Kona	oven-dried	0.55	0.43	1.08	0.17	2.22	6.44
40	Yellow Caturra	Kunia	oven-dried	0.51	0.43	1.29	0.14	2.37	6.75
41	Yellow Caturra	Maui	oven-dried	0.46	0.43	1.08	0.14	2.11	8.52
42	Yellow Caturra	Kona	sun-dried	0.58	0.47	1.17	0.09	2.31	7.76
43	Yellow Caturra	Maui	sun-dried	0.51	0.49	1.32	0.09	2.42	6.99

	3-CQA	4-CQA	5-CQA	5-FQA	3,4-diCQA	3,5-diCQA	4,5-diCQA
1	0.63	0.92	4.29	0.36	0.12	0.14	0.21
2	0.60	0.89	4.56	0.52	0.16	0.26	0.27
3	0.76	1.04	5.20	0.58	0.22	0.30	0.38
4	0.65	0.90	4.99	0.53	0.22	0.32	0.34
5	0.63	0.89	4.32	0.39	0.13	0.18	0.22
6	0.72	1.03	4.51	0.55	0.15	0.19	0.22
7	1.03	1.57	6.15	0.75	0.37	0.33	0.43
8	1.23	1.84	7.44	0.76	0.31	0.28	0.45
9	0.60	0.90	3.84	0.45	0.12	0.15	0.20
10	0.63	0.92	4.29	0.36	0.12	0.14	0.21
11	0.35	0.61	3.93	0.32	0.08	0.12	0.15
12	0.61	0.87	4.59	0.47	0.13	0.21	0.21
13	0.47	0.68	3.52	0.44	0.09	0.16	0.14
14	0.75	1.04	4.77	0.36	0.17	0.18	0.24
15	0.86	1.32	6.41	0.99	0.11	0.16	0.20
16	0.87	1.26	6.52	0.61	0.22	0.37	0.42
17	0.45	0.72	3.53	0.33	0.11	0.16	0.20
18	0.40	0.63	3.62	0.34	0.11	0.16	0.21
19	0.30	0.52	3.64	0.31	0.08	0.26	0.23
20	0.39	0.64	3.45	0.28	0.09	0.11	0.17
21	0.54	0.81	5.27	0.51	0.12	0.29	0.22
22	0.41	0.59	4.66	0.46	0.11	0.32	0.21
23	0.49	0.75	3.48	0.32	0.11	0.14	0.15
24	0.62	0.74	3.90	0.49	0.11	0.14	0.17
25	0.94	1.33	6.01	0.51	0.25	0.31	0.31
26	1.00	1.54	6.58	0.62	0.31	0.28	0.42
27	0.70	1.02	4.27	0.43	0.17	0.20	0.20
28	0.66	0.97	4.69	0.39	0.20	0.28	0.28
29	0.67	0.96	4.66	0.54	0.21	0.26	0.34
30	0.31	0.61	4.27	0.37	0.09	0.38	0.28
31	0.65	0.95	4.23	0.37	0.18	0.18	0.27
32	0.63	0.90	4.92	0.43	0.21	0.28	0.32
33	0.70	1.13	6.52	0.56	0.35	0.59	0.50
34	0.34	0.59	4.76	0.30	0.10	0.45	0.23
35	0.89	1.36	6.97	0.54	0.20	0.29	0.40
36	0.67	1.01	4.00	0.42	0.17	0.20	0.24
37	0.68	1.01	4.54	0.44	0.17	0.20	0.24
38	0.43	0.68	3.53	0.37	0.10	0.12	0.17
39	0.39	0.62	4.12	0.35	0.13	0.31	0.31
40	0.50	0.77	3.21	0.31	0.21	0.19	0.25
41	0.52	0.82	4.21	0.43	0.08	0.12	0.12
42	0.41	0.60	4.71	0.42	0.13	0.45	0.28
43	0.46	0.79	4.10	0.44	0.14	0.25	0.19

	total monoCQA	total diCQA	total CQA	total CGA	Molar ratio
1	5.85	0.47	6.32	6.68	18.24
2	6.05	0.69	6.74	7.26	12.80
3	6.99	0.90	7.89	8.47	11.33
4	6.54	0.88	7.42	7.94	10.86
5	5.85	0.53	6.38	6.77	15.96
6	6.26	0.56	6.82	7.36	16.40
7	8.74	1.13	9.87	10.61	11.32
8	10.51	1.04	11.56	12.32	14.71
9	5.35	0.47	5.82	6.27	16.72
10	5.85	0.47	6.32	6.68	18.24
11	4.88	0.35	5.23	5.56	20.36
12	6.08	0.56	6.64	7.10	15.91
13	4.67	0.39	5.06	5.50	17.65
14	6.56	0.59	7.16	7.51	16.10
15	8.59	0.47	9.06	10.05	26.60
16	8.65	1.02	9.67	10.27	12.40
17	4.69	0.47	5.17	5.50	14.41
18	4.65	0.49	5.14	5.48	13.94
19	4.45	0.56	5.01	5.33	11.55
20	4.49	0.37	4.86	5.14	17.57
21	6.61	0.63	7.24	7.75	15.34
22	5.67	0.64	6.30	6.76	12.99
23	4.72	0.39	5.11	5.43	17.43
24	5.26	0.43	5.69	6.17	17.83
25	8.28	0.87	9.16	9.67	13.82
26	9.13	1.01	10.14	10.76	13.19
27	6.00	0.57	6.57	7.01	15.34
28	6.31	0.76	7.07	7.46	12.14
29	6.29	0.81	7.10	7.64	11.34
30	5.19	0.75	5.94	6.31	10.12
31	5.83	0.62	6.46	6.83	13.63
32	6.44	0.81	7.25	7.67	11.63
33	8.35	1.44	9.80	10.35	8.43
34	5.69	0.78	6.47	6.77	10.63
35	9.23	0.89	10.12	10.66	15.19
36	5.68	0.61	6.29	6.71	13.50
37	6.23	0.60	6.83	7.28	15.06
38	4.63	0.39	5.01	5.39	17.48
39	5.13	0.75	5.88	6.22	10.02
40	4.48	0.64	5.12	5.43	10.12
41	5.54	0.31	5.85	6.28	25.98
42	5.72	0.86	6.58	7.00	9.68
43	5.34	0.58	5.92	6.36	13.32

	Dry Aroma	Wet Aroma	Acidity	Flavor	Body	Breeding Code
1	2.80	2.40	2.00	2.40	2.40	OA12C
2	2.61	2.50	1.46	2.54	1.89	OA15-11
3	2.57	2.82	1.96	2.77	2.04	OA12C
4	2.43	2.64	1.11	2.69	2.08	OA13C
5	2.57	2.68	1.21	2.65	2.15	OA14C
6	3.00	2.80	1.27	2.60	1.90	5175-6
7	3.13	2.53	1.30	2.73	1.90	5175-7
8	2.93	2.73	1.20	2.79	1.97	8667-5
9	2.67	2.63	1.43	2.73	1.87	8667-6
10	3.00	2.50	2.30	2.50	2.20	
11	2.70	2.40	2.40	2.90	2.30	
12	3.30	2.60	1.33	2.92	2.03	MA1-13
13	2.90	2.67	1.47	2.54	2.10	MA1
14	3.40	2.93	1.45	2.79	2.23	MA1-12
15	3.13	2.80	1.77	3.23	2.23	MA9-11
16	2.89	2.53	1.40	2.75	2.27	MA9
17	3.00	2.80	2.60	3.10	2.40	
18	3.30	2.70	2.00	2.70	2.20	
19	2.60	2.30	3.20	2.60	2.20	
20	3.40	2.70	1.90	2.90	2.30	
21	2.90	2.57	1.50	2.83	2.89	
22	3.20	2.63	2.13	2.43	2.71	
23	3.07	2.57	1.33	2.68	2.83	MA-6
24	3.10	2.67	1.50	2.70	2.79	KA19-C
25	3.03	2.57	1.63	2.85	2.93	MO21C
26	3.03	2.77	1.63	3.10	2.97	MO24
27	3.20	2.47	1.40	3.10	2.33	MO30
28	2.60	2.80	2.00	2.80	2.60	
29	2.70	2.40	1.70	2.70	2.60	
30	2.60	2.60	2.60	2.50	2.20	
31	2.70	2.60	2.10	2.70	2.50	
32	2.80	2.90	2.30	2.50	2.30	
33	2.77	2.62	1.79	2.71	1.97	
34	2.79	2.38	1.88	2.88	1.88	
35	2.91	2.50	1.41	2.84	2.12	OA33-1
36	2.82	2.47	1.53	2.81	2.06	KO32-C
37	3.00	2.67	1.30	3.00	2.07	KA18-C
38	3.10	2.70	1.70	2.70	2.50	
39	2.60	2.50	3.10	2.60	2.00	
40	3.00	2.60	1.80	2.70	2.50	
41	2.90	2.60	2.10	2.50	2.20	
42	2.67	2.13	1.70	2.53	1.82	
43	3.03	2.57	1.20	2.87	2.18	

Explanations and abbreviations

All values are percent dry weight basis of green coffee

BMxM = Mokka x Blue Mountain hybrid

CIxM = Mokka x Red Catuai hybrid

Total Acid = The sum of quinic, malic, citric and lactic acids

CQA = Caffeoylquinic acid

FQA = Feruloylquinic acid

CGA = Chlorogenic acid

Total monoCQA = The sum of 3-CQA, 4-CQA and 5-CQA

Total diCQA= The sum of 3,4-diCQA, 3,5-diCQA and 4,5-diCQA

Total CQA = The sum of monoCQA and diCQA

Total CGA = The sum of total CQA and 5-FQA

Molar Ratio = The molar ratio of monoCQA/diCQA

Breeding Code = the code used in the Hawai'i Agriculture Research Center's breeding program (Nagai et al., 2001). All other coffees were from the Hawai'i State Coffee Trial (Farmer's Bookshelf, 2002)

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